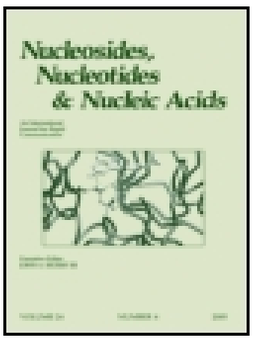

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Synthesis and cytotoxic activity of novel acyclic nucleoside analogues with functionality in click chemistry

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ABSTRACT

We describe synthesis of novel acyclic nucleoside analogues which are building blocks for CuAAC reaction and their activity against two types of human cancer cell lines (HeLa, KB). Three of chosen compounds show promising cytotoxic activity. Synthesis pathway starting from simple and easily accessible substrates employing DMT or TBDPS protective groups is described. Adenosine and thymidine analogues containing alkyne moiety and adenosine analogue containing azido group were synthesized. The obtained units showed ability of forming triazole motif under the CuAAC reaction conditions.

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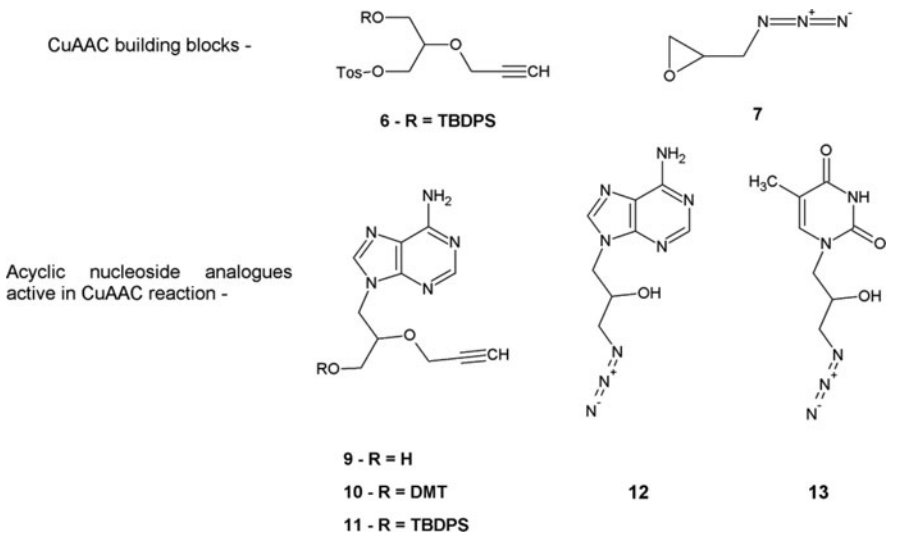
GRAPHICAL ABSTRACT



Introduction

Thanks to DNA sequencing technology precise targeting of pathogens become easier. Antisense oligonucleotides can inhibit expression of particular proteins by

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hybridization to specific mRNAs. It can result in therapeutic effect exhibited as considerable slowing down or even stopping the replication process of viruses or uncontrolled growth of cancer cells. Oligonucleotide antisense therapy is a complex strategy not only because of necessity of targeting at exactly specified sequence but also because it needs introducing structural modifications to oligonucleotide drug molecule. Unmodified oligonucleotides are efficiently degraded by endogenous DNase enzymes but chemical modification of the phosphodiester backbone can give them stability in cell media. Common modifications used in analogues of this type are (among others) phosphorothioates, methyl phosphonates and phosphoramidates.[1–3] Introducing modification in sugar moiety of nucleoside analogues may also have impact on enzymatic stability of oligonucleotide chain. Incorporation of well-known locked nucleic acid units (LNAs) into antisense DNA oligonucleotides provides unprecedented increase of duplexes thermodynamic stability and greater stability to nucleases.[4] While incorporation of unlocked nucleic acid units (UNAs) has destabilizing effect on duplexes but also improves biological resistance of oligonucleotides.[5] This phenomenon can be used to manipulate the thermodynamic properties of DNA and RNA probes.

Other efficient approach is to use oligonucleotide analogues with linkage between nucleoside units different from phosphodiester. Peptide Nucleic Acids are neutral molecules lacking of a negatively charged backbone which contributes to increased stability.[6,7] This characteristic is shared with Triazole-Linked DNA oligonucleotides synthesized with the use of click chemistry.[8–10] This kind of structures can be read through by DNA and RNA polymerases which suggests, that triazole is appropriate linker for ligation of oligonucleotides to form very long strands of DNA. There are also known examples of shorter analogues containing only triazole in place of phosphodiester linkages between nucleosides.[11] So far it was possible to synthesize TL-DNA (triazole linked DNA) decamer built only with thymidine moieties. Isobe group used microwave irradiation as an effective catalyst in the synthetic procedure.[12] In other paper the relation between the length of triazole internucleotide linkages and duplex stability was analysed.[13] Most stable and favourable are rigid connections of six bonds length.

It is also known that many different types of nucleoside analogues are effective therapeutics in antivirus and anticancer therapy. Some of the most potent and widely used drugs are nucleoside analogues having azido moiety or acyclic structure. Acyclovir (ACV) was discovered in 1970's and since then is used as a very efficient antivirus agent (against HSV).[14] It is also possible to turn ACV into an anticancer drug by nanoparticle delivery directly to the cancer cells in the form of monophosphate.[15] Cidofovir and adefovir are similar nucleoside analogues based on cytosine or adenosine and acyclic sugar mimic. All of these compounds show high antiviral activity.[16]

Other widely used antivirus drug is azidothymidine which was primarily designed as a compound with anticancer activity.[17] Mechanism of its action involves its intracellular conversion to the 5′-triphosphate (via the corresponding mono-and diphosphate) and is dependent on azido moiety present in the 3′ position

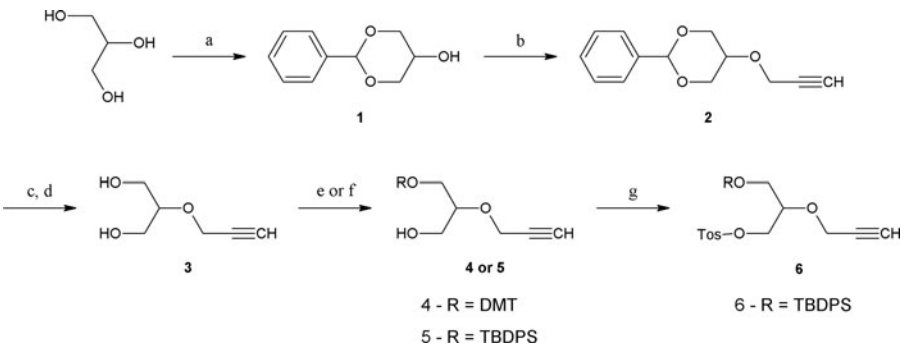
of sugar part. It acts as a competitive inhibitor of DNA polymerases and a chain terminator of the nascent DNA strand due to the lack of a 3′-hydroxyl group.[18] Antibacterial and antiviral activity of AZT was discovered in 1980's and since then zidovudine was the first nucleoside analogue licensed for the treatment of HIV infection.[16,19] Many derivatives of AZT showed antiviral activity and some of them even demonstrate improved activity.[20] 3′-Azido group of AZT is not only the source of antiviral activity but also constitutes an excellent substrate for modifications via "click" reactions.[21–25] Click chemistry was established as the powerful tool in medicinal chemistry giving wide perspectives for nucleosides modifications and drug design.[26,27]

Similarity in structure of our building blocks to above described antivirus drugs with anticancer potential, prompted us to test biological activity of our final products and intermediates. The tests were performed regarding their anticancer activity against HeLa and KB cells. In our letter we describe synthetic route of novel building blocks bearing azido or alkyl moiety active in CuAAC reaction leading to triazole-linked dinucleotides analogues synthesis. The comparison of biological activity of our by-products and final building blocks is also presented.

Results and discussion

We decided to undertake synthesis of modified dinucleotide fragments connected via triazole linker containing acyclic sugar analogue. For this purpose we designed acyclic building blocks having alkyne or azido moieties active in Copper-Catalyzed Huisgen Azide-Alkyne 1,3-Dipolar Cycloaddition Reaction (CuAAC).[28,29]

The project was started with an idea of using as simple substrates as possible. In order to obtain final analogues of dinucleotides, synthesis was divided in two paths leading to alkyne or azido building blocks. The first part of our work was based on known modification of glycerol.[30] This molecule was meant to imitate sugar part in acyclic analogues of nucleosides. Whole procedure of synthesis of building block with alkyl group is shown in Scheme 1. In the first step we had to protect two of three



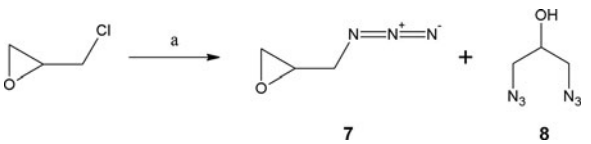
Scheme 1. Synthesis of alkyl building block. Reagents and conditions: (a) benzaldehyde, PTSA, reflux, 5h, 20%; (b) NaH, 0°C, DMF, propargyl bromide, 33%; (c) 90% formic acid, 4h; (d) 25% NH_4OH, 12h; (e) ACN, Et_3N, DMTCl, rT, 25%; (f) NaH, 0°C, TBDPSCl, 2h, 53%; (g) DMAP, 0°C, p-Toluenesulfonyl chloride, 3h, 83%.

hydroxyl groups of glycerol. Blocking of both primary OH groups was achieved by reaction with benzaldehyde and generation of cyclic acetal.[31,32] In the reaction mixture 1,2-substituted product dominated, but 1,3-protected compound **1** of our interest crystalized easily from the reaction mixture. Despite of the unfavorable yield (20%), due to simplicity of this reaction we achieved sufficient amount of product by simply working on bigger scale.

In the next step we decided to introduce alkyne group in 2-OH position in the reaction with propargyl bromide. Reaction resulted in substituted product **2** in a good yield. Subsequently benzylidene acetal protective group was cleaved in order to expose both primary hydroxyl groups to functionalization. Deprotection was performed in two steps, the first consisted of formic acid treatment, which resulted in mixture of mono and disubstituted formate esters. Then the second treatment with ammonia in methanol gave the desired product **3**. In the next step, we had to introduce protective group only in one of two equivalent primary hydroxyls. We decided to use sterically large protecting group to increase chances in blocking only one hydroxyl. First we tested DMT chloride as a protective reagent but the experiment resulted in two products, minority of which was desired monosubstituted derivative **4** accompanied by large amount of disubstituted compound. Subsequently, change of protection to TBDPS group led to better results. Disubstituted product was also formed, but monosubstituted compound **5** prevailed. Pure product **5** was isolated after simple column chromatography separation.

The last step towards alkyne building block was to place good leaving group on free primary hydroxyl position. By reaction with tosyl chloride product **6** was obtained in a good yield (83%). Compound **6** turned out to be a good partner for coupling with purine and pyrimidine bases.

In the following part of our work synthesis of the second building block having azido moiety was carried out. The aim was to obtain second active partner in Huisgen cycloaddition reaction.[28,29] Again we decided to simplify the whole procedure as much as possible, and final synthesis route is shown in Scheme 2. Preparation of sugar moiety acyclic mimic took only one step and started with epichlorohydrin. Substitution of chloride with azido group was efficient.[33] Problems arose with purification of monosubstituted product **7** having strained oxirane ring. Through the ring opening disubstituted azido by-product (**8**) was also formed. The reaction mixture was difficult to separate and it required two steps, in the first one mono- and diazido were separated via column chromatography, but the desired monoazido was accompanied by considerable amount of epichlorohydrin. In the second step we removed epichlorohydrin under reduced pressure. We decided to avoid distillation of crude reaction mixture as the low-molecular weight azido derivatives are prone



Scheme 2. Synthesis of azide building block. Reagents and conditions: (a) sodium azide, 4h, RT.

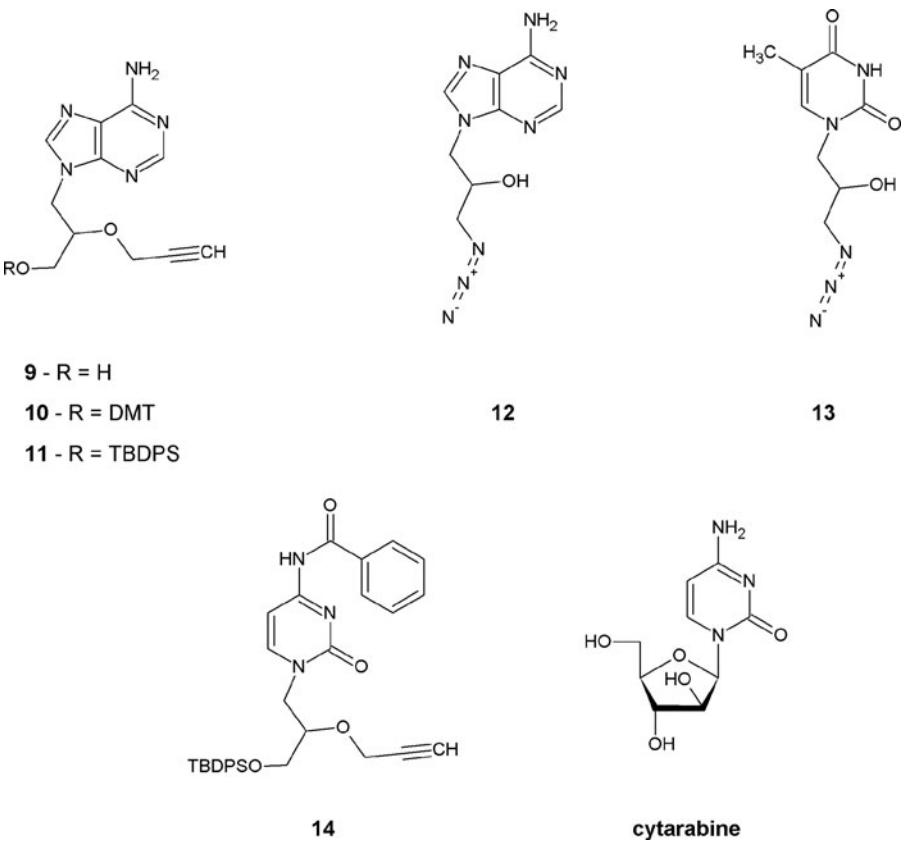


Figure 1. Structures of all synthesized nucleoside analogues and structure of cytarabine standard.

to explosion. Fortunately the desired azide-epichlorohydrin mixture was safe to distill.[33] The product distilled at the temperature 40–45°C (5 mm Hg). Product **7** is a good partner for coupling with different purine and pyrimidine bases.

As the last synthetic step the ability of our building blocks to couple with DNA bases was tested. We compared efficiency of reactions conducted in the standard conditions and under microwave irradiation. Standard conditions means heating of reaction mixture to 60°C for 72 hours in the presence of potassium carbonate as basic catalyst.[34] Method involving MV irradiation was performed in microwave reactor.[35] In the presence of K_2CO_3 as a catalyst and temperature rising to 140°C, the reaction occurred in much shorter time – completed in few hours, but the coupling yield didn't change meaningfully. The solvent in both procedures was DMF. Figure 1 shows structures of acyclic nucleoside analogues synthesized in our project. All of them have azido or alkyne groups, active in CuAAC reaction.

In conclusion of synthetic part of our project we synthetized alkyne analogue of adenosine and two azido nucleoside analogues – with adenine and thymine. The products showed activity in CuAAC reaction which resulted in high yield of triazole-linked dinucleotide formation.[36] Product **14** having cytosine as a nucleic base in alkyne series was received only in small amount and was not employed in CuAAC reaction. Guanosine analogue was not formed with any of the above methods.

Table 1. Cytotoxic activity of selected compounds.

Compound	Cytotoxicity (IC_{50}, μg/mL)[a] ± SD[b]		
	HeLa	KB	HDF
6	4.27 ± 0.47	4.16 ± 0.04	7.02 ± 0.81
7	29.16 ± 1.73	28.51 ± 1.60	39.20 ± 0.93
9	17.21 ± 0.33	17.90 ± 0.03	25.10 ± 0.02
10	1.03 ± 0.04	0.97 ± 0.21	2.82 ± 0.07
11	1.60 ± 0.89	1.42 ± 3.19	2.70 ± 0.58
12	7.36 ± 1.23	7.91 ± 1.09	11.82 ± 0.02
13	2.11 ± 0.29	2.49 ± 10.26	3.84 ± 2.56
14	1.52 ± 0.20	1.39 ± 0.36	3.17 ± 0.04
Cytarabine	0.81 ± 3.92	0.93 ± 4.25	2.75 ± 0.20

[a]IC_{50} – half maximal inhibitory concentration.
[b]SD – standard deviation of three independent experiments.

In the last part of our project we performed biological activity analysis. From the pool of synthesized compounds eight were chosen, which showed structural similarity to known antivirus agents. Tests were performed on human cancer cells HeLa (cervical cancer cell line) and KB (carcinoma nasopharynx), compared to HDF cells (Human Dermal Fibroblasts). Details of cell cultures preparation and tests performance are presented in Materials and methods section of this manuscript.

Results are shown in Table 1. Three compounds i.e. **10**, **11**, **14** showed higher activity than cytarabine standard. Their common feature is presence of sterically big protective groups (TBDPS or DMT). Presence of this protection changes character of compounds to more lipophilic, which is probably the reason of better transport through cell membrane and therefore higher anticancer activity.[37] The highest activity (IC_{50}) with satisfactory selectiveness (SI) has compound **10**. Compounds having alkyne group seem to be slightly more active than compounds with azido group.

Conclusion

In summary, we report the successful synthesis of adenosine and thymidine acyclic analogues containing alkyl moiety as well as adenosine acyclic analogue containing azido moiety. All products show activity in CuAAC reaction and triazole formation. Cytotoxic activity analysis of selected compounds revealed connection between structural similarity of compounds **10**, **11**, **14** and their high efficiency. Our results suggest that synthetized nucleoside analogues can be used effectively in TL dinucleotide formation and further oligonucleotide synthesis.

Materials and methods

General remarks

Specific procedures for the preparation of the products **1–7** as well as general procedure for the acyclic nucleosides analogues synthesis (**9–14**) are given below. Characterization data are included. Dichloromethane, chloroform, pyridine and

DMF were purified before use according to common procedures.[38] All the other solvents and reagents were used without purification as acquired from commercial sources. Progress of the chemical reactions was monitored by thin-layer chromatography on silica gel 60-F254 aluminum plates and detected under UV light. All NMR spectra were recorded employing a Spectrometer Varian VNMR-S 400 MHz, and chemical shifts (δ) are in ppm relative to TMS as internal standard. Waters Micromass ZQ (ESI ionisation) or Waters Maldi Q-Tof Premiere (MALDI ionisation) were used for the mass spectra measurements.

Synthetic procedures

Synthesis of 2-Phenyl-1,3-dioxan-5-ol (1)

Freshly distilled benzaldehyde (53 g; 0.5 mol) was dissolved in 50 mL of toluene and anhydrous glycerin (55.3 g; 0.6 mol) in 50 mL of toluene was added. To the mixture was added p-toluenosulfonic acid (2 g; 0.01 mol) and reflux with Dean-Stark apparatus was carried out for 5 hours. The reaction mixture was cooled down to room temperature and neutralized with 4M potassium hydroxide solution. The organic layer was washed twice with water and once with saturated $NaHCO_3$ aq. The organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated. Pure product was crystalized from cooled mixture of hexane and toluene (5:3, v/v) to give 20 g of product (20% yield).

Compound (**1**) is well known in literature, NMR spectral analysis confirmed the structure.[31]

Synthesis of 2-phenyl-5-(prop-2-ynyl-1-oxy)-1,3-dioxan (2)

Compound **1** (2.1 g; 12 mmol) was dissolved in 15 mL of DMF and the mixture was cooled down to 0°C. Then sodium hydride (0.42 g; 18 mmol) was added in few small portions (avoiding rise of temperature). After stirring for 30 min propargyl bromide was added (1.73 g; 15 mmol) and reaction was carried out at room temperature for 12 hours. Progress of the reaction was controlled by TLC (toluene-AcOEt 7:3, v/v). Reaction was quenched with 70 mL of water and extracted with chloroform (3 × 30 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography (CH_2Cl_2:AcOEt 1:1 + MeOH 1.5%), giving compound **2** (0.84 g; 33.1%).

NMR ^1H ($CDCl_3$, δ, ppm): 2.43 (m, 1H, CCH), 3.65 (quintet, J = 1.7 Hz, 1H, H-5), 4.09 (dd, J_1 = 12.9 Hz, J_2 = 1.6 Hz, 2H, OC**H**$_2$CCH), 4.37 (m, 4H, H-4, 4′,H-6, 6′), 5.56 (s, 1H, H-2), 7.35 (m, 3H, phenyl), 7.50 (m, 2H, phenyl)

NMR ^{13}C ($CDCl_3$, δ, ppm): 55.51; 68.63; 74.85; 77.20; 79.34; 101.30; 126.06; 128.15; 128.88; 138.04

ESI-MS, calculated for $C_{13}H_{14}O_3$ – m/z 218, found – m/z 217 (M^+-H)

Synthesis of 2-(prop-2-ynyl-1-oxy)-propan-1,3-diol (3)

Compound **2** (5.73 g; 26 mmol) was dissolved in 15 mL of 90% formic acid. Solution was stirred for 4 hours at room temperature. Progress of the reaction was controlled

by TLC (toluene-AcOEt 7:3, v/v). Mixture was diluted with water and extracted three times with 10 mL portions of hexane. Water layer was evaporated to dryness. The residue was placed in 10 mL of methanol and 5 mL of ammonia aq was added. Reaction was carried out for 12 hours at room temperature. Progress of the reaction was controlled by TLC (toluene-AcOEt 7:3, v/v). The reaction mixture was evaporated to dryness giving 3.71 g of crude product, which was used further without purification.

NMR ^{1}H ($CDCl_3$, δ, ppm): 1.34 (t, J = 5.4 Hz, 2H, OH), 2.93 (t, J = 2.9 Hz, 1H, C≡CH), 3.53–3.31 (m, 5H, H-1, H-1′, H-2, H-3,H-3′), 4.11 (d, J = 3.1 Hz, 2H, OC**H**$_2$C≡CH)

ESI-MS, calculated for $C_6H_{10}O_3$ – m/z 130, found – m/z 131 ($M + H^+$)

Synthesis of 3-dimethoxytrityl-2-(prop-2-ynyl-1-oxy)-propan-1-ol (4)

Compound **3** (0.5 g; 3.84 mmol) was evaporated with ACN (3 × 10 mL). Substrate was dissolved in 30 mL of anhydrous ACN and triethylamine was added (0.4 g; 3.84 mmol). Then dimethoxytrityl chloride was added (1.3 g; 3.84 mmol) and reaction was carried out at room temperature for 12 hours. Progress of the reaction was controlled by TLC (toluene-AcOEt 7:3, v/v) and visualized by spraying with 10% sulfuric acid in ethanol and heating. Mixture was concentrated with evaporator. Residue was dissolved in CH_2Cl_2 and washed with 45 mL of saturated $NaHCO_3$ aq (3 × 15 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography (CH_2Cl_2:hexane 1:1 + MeOH 1%), to give pure compound **4** (0.41 g; 25%).

NMR ^{1}H ($CDCl_3$, δ, ppm): 2.43 (t, J = 2.3 Hz, 1H, CCH), 3.30–3.16 (m, 2H, H-1, 1′), 3.78–3.58 (m, 4H, OH, H-3, 3′, H-2), 3.79 (s, 6H, OCH_3), 4.24 (dd, J_1 = 15.8 Hz, J_2 = 2.4 Hz, 1H, OC**H**$_2$C≡CH), 4.34 (dd, J_1 = 15.8 Hz, J_2 = 2.4 Hz, 1H, OC**H**$_2$C≡CH), 7.47–6.76 (m, 13H, trityl)

ESI-MS, calculated for $C_{27}H_{28}O_5$ – m/z 432, found – m/z 455 ($M + Na^+$)

Synthesis of 2-(prop-2-ynyl-1-oxy)-3-(tert-butyldiphenylsilyl)-propan-1-ol (5)

Compound **3** (1.17 g; 9 mmol) was dissolved in 10 mL of THF and the mixture was cooled down to 0°C. Then sodium hydride (0.216 g; 9 mmol) was added in few small portions (avoiding rise of temperature). After stirring for 30 min *tert*-butyldiphenylsilyl chloride was added (2.47 g; 9 mmol) and reaction proceeded at room temperature for 2 hours. Progress of the reaction was controlled by TLC (toluene-AcOEt 7:3, v/v). Reaction mixture was poured to 30 mL of diethyl ether and washed twice with 10% K_2CO_3 aq (2 × 15 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography ($CHCl_3$:hexane 1:3 + MeOH 3%), to give pure monosubstituded compound **5** (1.75 g; 53%).

NMR ^{1}H ($CDCl_3$, δ, ppm): 1.06 (s, 9H, t-butyl), 2.06 (ddd, J_1 = 6.8 Hz, J_2 = 4.8 Hz, J_3 = 1.2 Hz, 1H, OH), 2.41 (t, J = 2.4 Hz, 1H, CCH), 3.84–3.62 (m, 5H, H-1, H-1′, H-2, H-3, H-3′), 4.19 (dd, J_1 = 15.9 Hz, J_2 = 2.4 Hz 1H, H of OC**H**$_{2a}$CCH), 4.28

(dd, $J_1 = 15.9$ Hz, $J_2 = 2.4$ Hz 1H, H' of OC**H**$_{2b}$CCH), 7.49–7.34 (m, 6H, phenyl), 7.72–7.63 (m, 4H, phenyl)

NMR ^{13}C ($CDCl_3$, δ, ppm): 19.13; 26.77; 57.56; 62.66; 63.41; 74.48; 79.41; 79.94; 127.75; 129.80; 133.07; 135.53

ESI-MS, calculated for $C_{22}H_{28}O_3Si$ – m/z 368, found – m/z 391 ($M + Na^+$)

Synthesis of 2-(prop-2-ynyl-1-oxy)-3-(tert-butyldiphenylsilyl)-1-tosyloxypropane (6)

Compound **5** (1.75 g; 4.75 mmol) was dissolved in 20 mL of distilled CH_2Cl_2 and DMAP (1.76 g; 14.25 mmol) was added. The mixture was stirred and cooled down to 0°C. Then tosyl chloride (1.83 g; 9.5 mmol) was added. Reaction was carried out for 3 hours, allowed to warm up to room temperature. Progress of the reaction was controlled by TLC (toluene-AcOEt 7:3, v/v). Reaction mixture was diluted with CH_2Cl_2 and washed twice with water and once with saturated $NaHCO_3$ aq. The organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography (CH_2Cl_2:hexane 1:3 + AcOEt 1.5%), to give pure compound **6** (2.06 g; 83.1%).

NMR ^{1}H ($CDCl_3$, δ, ppm): 0.99 (s, 9H, t-butyl), 2.32 (t, J = 2.4 Hz, 1H, CCH), 2.42 (s, 3H, C**H**$_3$-Tos), 3.61 (dd, $J_1 = 10.8$ Hz, $J_2 = 6.2$ Hz, 1H, H-3), 3.70 (dd, $J_1 = 10.8$ Hz, $J_2 = 4.9$ Hz, 1H, H-3′), 3.80 (m, 1H, H-2), 4.11 (m, 3H, OC**H**$_2$CCH, H-1), 4.27 (dd, $J_1 = 10.4$ Hz, $J_2 = 3.9$ Hz, 1H, H-1′), 7.82–7,26 (m, 14H, phenyl)

NMR ^{13}C ($CDCl_3$, δ, ppm): 19.09; 21.63; 26.67; 57.61; 62.08; 69.25; 74.65; 76.44; 77.20; 79.26; 127.73; 127.99; 129.80; 132.91; 135.51; 144.76

ESI-MS, calculated for $C_{29}H_{34}O_5SSi$ – m/z 522, found – m/z 545 ($M + Na^+$)

Synthesis of azidomethyloxirane (7)

Epichlorohydrin (50 g; 0.54 mmol) was mixed with 100 mL of water and stirred vigorously with magnetic stirrer. Then solution of NaN_3 (39 g; 0.6 mmol) in 140 mL of water was added slowly and in small portions, during 3 hours time, and then stirred for 30 min more. Bottom layer was separated and upper layer was extracted with three portions of CH_2Cl_2 (3 × 60 mL). Extracts and bottom layer were combined, dried over anhydrous $MgSO_4$, filtered and evaporated. The residue was purified by SiO_2 column chromatography (CH_2Cl_2 + MeOH 2.5%), to give fraction containing monosubstituted product, which was further separated by distillation under reduced pressure.

Compound (**1**) is known in literature, spectral analysis confirmed structure.[39]

General procedures for synthesis of nucleosides analogues (9–14)

With classical method

All of used nucleic bases and K_2CO_3 were dried under reduced pressure (2 mm Hg) for 2–3 hours. Substrate with alkyne moiety was also dried under reduced pressure, substrate with azido functionality was used immediately after distillation.

Purine or pyrimidine base was used in the molar excess as a 2 equivalents relative to the substrate. Compounds were suspended in 10–20 mL of dry DMF and 200 mg of K_2CO_3 was added. Reaction was carried out on magnetic stirrer at the temperature 60°C for 72 hours. Progress of the reaction was controlled by TLC (chloroform-methanol 9:1, v/v). Reaction mixture was filtered and evaporated to dryness. Crude product was purified by column chromatography (CH_2Cl_2 + MeOH 3%).

With MV irradiation

All of used nucleic bases and K_2CO_3 were dried under reduced pressure (2 mm Hg) for 2–3 hours. Substrate with alkyne moiety was also dried under reduced pressure, substrate with azido functionality was used immediately after distillation.

Purine or pyrimidine base was used in the molar excess as a 2 equivalents relative to the substrate. Compounds were suspended in 10–20 mL of dry DMF and 200 mg of K_2CO_3 was added. Reaction was carried out in microwave reactor (MARS 6 – CEM corporation) in few cycles following conditions:

- temperature rise to 140°C in 1 minute time,
- the temperature was held for 30 minutes and then reaction was cooled down to room temperature

Progress of the reaction was controlled by TLC (chloroform-methanol 9:1, v/v). Reaction cycles were repeated 8 to 10 times which gave total time 4–5 hours. Reaction mixture was filtered and evaporated to dryness. Crude product was purified by column chromatography (CH_2Cl_2 + MeOH 3%).

Spectral data of acyclic nucleosides products

Compound 9. NMR ^{1}H (D_2O, δ, ppm): 2.37 (t, J = 2.4 Hz, 1H, CCH), 3.67 (dd, J_1 = 12.4 Hz, J_2 = 4.8 Hz, 1H, H-3) 3.85 (dd, J_1 = 12.4 Hz, J_2 = 4.0 Hz, 1H, H-3′), 4.44–4.00 (m, 5H, H-1, H-1′, H-2, OC**H$_2$**CCH), 8.18 (s, 1H, H-8 adenine), 8.53 (s, 1H, H-2 adenine)

ESI-MS, calculated for $C_{11}H_{13}N_5O_2$ – m/z 247, found – m/z 270 ($M + Na^+$), m/z 246 (M^+-H), m/z 282 ($M + Cl^-$)

Compound 10. NMR ^{1}H ($CDCl_3$, δ, ppm): 2.14 (t, J = 2.4 Hz, 1H, CCH), 3.20 (dd, J_1 = 10.4 Hz, J_2 = 4.7 Hz, 1H, H-3), 3.30 (dd, J_1 = 10.4 Hz, J_2 = 4.4 Hz, 1H, H-3′), 3.80 (s, 6H, OCH_3), 4.15–3.93 (m, 2H, H-2, OC**H$_2$**CCH), 4.35–4.14 (m, 2H, H-1, OC**H$_2$**CCH), 4.47 (dd, J_1 = 14.4 Hz, J_2 = 3.4 Hz, 1H, H-1′), 5.67 (s, 2H, NH_2), 7.00–6.71 (m, 4H, phenyl), 7.57–7.15 (m, 9H, phenyl), 7.90 (s, 1H, H-8 adenine), 8.33 (s, 1H, H-2 adenine)

NMR ^{13}C ($CDCl_3$, δ, ppm): 45.14; 55.22; 57.62; 62.71; 74.53; 76.11; 78.91; 86.46; 113.19; 126.90; 127.90; 128.04; 129.97; 135.66; 141.83; 144.50; 152.75; 155.21; 158.55

ESI-MS, calculated for $C_{32}H_{31}N_5O_4$ – m/z 549, found – m/z 584 ($M + Cl^-$), m/z 621 ($M + Br^-$)

Compound 11. NMR ^{1}H (DMSO-d_6, δ, ppm): 1.00 (s, 9H, t-butyl), 3.25 (t, J = 2.4 Hz, 1H, CCH), 3.70 (m, 2H, H-1, H1′), 4.22–4.01 (m, 3H, H-2, OC**H$_2$**CCH),

4.28 (dd, $J_1 = 14.3$ Hz, $J_2 = 7.3$ Hz, 1H, H-3), 4.40 (dd, $J_1 = 14.3$ Hz, $J_2 = 4.4$ Hz, 1H, H-3′), 7.18 (s, 2H, NH_2), 7.67–7.36 (m, 10H, phenyl), 8.03 (s, 1H, H-8 adenine), 8.14 (s, 1H, H-2 adenine)

NMR ^{13}C (DMSO-d_6, δ, ppm): 18.78; 26.58; 43.59; 56.88; 63.32; 76.77; 77.01; 79.90; 118.52; 127.89; 129.90; 132.53; 135.06; 141.19; 149.71; 152.38; 155.91

ESI-MS, calculated for $C_{27}H_{31}N_5O_2Si$ – m/z 485, found – m/z 486 ($M + H^+$), m/z 508 ($M + Na^+$)

Compound 12. NMR ^1H (DMSO-d_6, δ, ppm): 3.24 (dd, $J_1 = 12.7$ Hz, $J_2 = 5.9$ Hz, 1H, H-3), 3.36 (m, 1H, H-3′), 4.10 (m, 2H, H-1, H-1′), 4.20 (m, 1H, H-2), 5.65 (d, J = 5.1 Hz, 1H, OH), 7.21 (s, 2H, NH_2), 8.05 (s, 1H, H-8 adenine), 8.14 (s, 1H, H-2 adenine)

ESI-MS, calculated for $C_8H_{10}N_8O$ – m/z 234, found – m/z 235 ($M + H^+$), m/z 233 (M^+-H), m/z 269 ($M + Cl^-$)

Compound 13. NMR ^1H (DMSO-d_6, δ, ppm): 1.75 (d, J = 1.2 Hz, 3H, CH3), 3.39–3.15 (m, 2H, H-3, H-3′), 3.48 (dd, $J_1 = 13.7$ Hz, $J_2 = 8.3$ Hz, 1H, H-1), 3.75 (dd, $J_1 = 13.7$ Hz, $J_2 = 3.9$ Hz, 1H, H-1′), 3.91 (m, 1H, H-2), 5.56 (d, J = 5.6 Hz, 1H, OH), 7.4 (q, J = 0.9 Hz, 1H, H-6 thymine), 11.23 (s, 1H, NH)

ESI-MS, calculated for $C_8H_{11}N_5O_3$ – m/z 225, found – m/z 248 ($M + Na^+$), m/z (264 ($M + K^+$)

Compound 14. NMR ^1H ($CDCl_3$, δ, ppm): 1.08 (s, 9H, t-Bu); 2.25 (t, 1H, J = 2.4 Hz, CCH); 3.59 (dd, 1H, J = 13.5 Hz, J = 9.1 Hz, C**H_{2b}**-OSi); 3.76 (dd, 1H, J = 11.3 Hz, J = 4.1 Hz, CH_{2b}-N); 3.81 (dd, 1H, J = 11.3 Hz, J = 4.1 Hz, CH_{2a}-N); 4.01 (dd, 1H, J = 16.0 Hz, J = 2.4 Hz, C**H_{2b}**CCH); 4.05 (m,1H, CH); 4.15 (dd, 1H, J = 16.0 Hz, J = 2.4 Hz, C**H_{2a}**CCH); 4.50 (dd, 1H, J = 13.5 Hz, J = 2.8 Hz, C**H_{2a}**-OSi); 7.92 -7.33 (m, 15 H, aromatic);

NMR ^{13}C ($CDCl_3$, δ, ppm): 162.3; 152.7; 150.7; 135.6; 135.5; 133.1; 132.9; 132.8; 129.9; 129.0; 127.8; 127.7; 127.5; 79.1; 76.2; 74.5; 63.3; 57.7; 52.0; 30.1; 26.8; 19.2.

MS (MALDI) calculated for $C_{33}H_{35}N_3O_4Si$ – $M^+ = 565$, found 566 ($M + H^+$); 588($M + Na^+$)

Biological activity analysis

Tests were performed on human cancer cells HeLa (cervical cancer cell line) and KB (carcinoma nasopharynx). Cells were cultured in RPMI 1640 medium. Each medium was supplemented with 10% fetal bovine serum, 1% L-glutamine and 1% penicillin/streptomycin solution. The cell lines were kept in the incubator at 37°C. The optimal plating density of cell lines was determined to be 5×10^4. All the cell lines were obtained from The European Collection of Cell Cultures (ECACC) supplied by Sigma-Aldrich.

The protein-staining sulforhodamine B (SRB, Sigma-Aldrich) microculture colorimetric assay, developed by the National Cancer Institute (USA) for in vitro antitumor screening was used in this study, to estimate the cell number by providing a sensitive index of total cellular protein content, being linear to cell density.[40] The monolayer cell culture was trypsinized and the cell count was adjusted to 5×10^4 cells. To each well of the 96 well microtiter plate, 0.1 mL of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was washed out and 100 µL of six different compound concentrations were added to the cells in microtitre plates. The tested compounds were dissolved in DMSO (20 µM) and the content of DMSO did not exceed 0.1%; this concentration was found to be nontoxic to the cell lines. The cells were exposed to compounds for 72 hours. After that, 25 µL of 50% trichloroacetic acid was added to the wells and the plates were incubated for 1 hour at 4°C. The plates were then washed out with the distilled water to remove traces of medium and next dried by the air. The air-dried plates were stained with 100 µL SRB and kept for 30 minutes at room temperature. The unbound dye was removed by rapidly washing with 1% acetic acid and then air dried overnight. The optical density was read at 490 nm. All cytotoxicity experiments were repeated three times. Cell survival was measured as the percentage absorbance compared to the control (non-treated cells). Cytarabine (Sigma-Aldrich) was used as the internal standard.

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